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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACT OF COFFEE HUSK

Microbiology

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ABSTRACT

In the present study methanolic extract of coffee husk was tested for phytochemical analysis and antimicrobial activity. The husk extract was examined by using standard qualitative phytochemical tests and the anti-microbial activity was done against five bacterial species of ***Streptococcus pyogenes, staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*** using agar well diffusion method. The results of phytochemical analysis showed that the presence of alkaloids, flavonoids, saponins, tannins, phenols and carbohydrates. The anti-microbial activity of husk extract showed higher inhibitory effect on gram negative bacteria (***E. coli and Klebsiella Pneumoniae***). The results confirmed that the coffee waste can be used therapeutically.

KEYWORDS

coffee husk, phytochemical analysis, anti-microbial analysis.

INTRODUCTION:

Coffee is one of the most popular beverages in the world and it has grown steadily in commercial importance over the last 150 years. Coffee generates large amount of coffee by-products/ residues during processing. Depending upon the method of coffee cherries processing different residues are obtained. Coffee husks are the major solid residue from the handling and processing of coffee, since for every 2kg of coffee beans produced, approximately 1kg of husks are generated. Mounjouenpoy Pauline, Ntoupka Mama and FalloJustin et al (2010). Adams MR, Dougan J et al (1987)

Coffee pulp and husk are the main by-products from coffee processing of the whole berry. It is normally left decomposed naturally to become a fertilizer used in the coffee field. This cause a lot of environmental problems as a result of its slow decomposition process. This effect appears to be due to the presence of the bioactive compounds in the coffee pulp, such as caffein, chlorogenic acid, flavonoids, anthocyanins and tannins Which are reported to process antimicrobial and antiphysiological activities. Pulp and husk are rich in carbohydrates, minerals, proteins and organic compounds. The byproduct utilization requires in urgent attention to the ecotoxicological and environmental impacts of coffee waste.

In India Karnataka and Andhra Pradesh are the most suitable area for Arabica coffee cultivation. In Andhra Pradesh Coffee is grown in the agency areas of Chintapalli, Paderu and Maredumilli of Visakhapatnam and East Godavari districts. The present study was aimed to investigate the phytochemical analysis and antimicrobial activity of Arabica Coffee husk extract.

MATERIALS AND METHODS:

Study area:

Chintapalli located at North Easter part of India between 17^{0}44'22"east to 82^{0}38'04". The temperature in the hill track village by deducting 2 to 30^{0}c recorded at the local agriculture research station, chintapalli. Lambasingi area is referred to Andhra Kashmir by sightseers as temperature dip as low as 0^{0}c during December and January Geetha et al (2016).

Collection of sample:

The Arabica Coffee waste was collected from chintapalli, Visakhapatnam. The collected waste was transferred to laboratory with in 24hrs. The sample was air dried under sunlight till the moisture content was removed. The dried sample was ground with blender to make fine powder.

Extraction:

The dried and powdered husk sample (150gm) was extracted with 200ml of each solvent separately by using a Soxhlet extractor for 2 to 5h at a temperature not exceeding the boiling point of the solvent. The solvent methanol was used for the extraction. The extract was distilled by the distillation unit and were transferred amber glass bottles and kept at 4^{o}c. The extract was dissolved in Dimethyle sulphoxide (DMSO) to prepare different concentrations (50µl, 100µl,150µl 200µl).

Phytochemical procedure

Test for Flavonoids:

2ml of extract was added with few drops of sodium hydroxide, formation of intense yellow colour was observed. To this few drop of 70% dilute hydrochloric acid were added and the yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for Alkaloids:

To 2ml of extract 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed. Appearance of dark orange or purple colour indicates the presence of alkaloids.

Test for Saponins:

To 2ml of extract, 6ml of distilled water was added and shaken vigorously; formation of bubbles or persistant foam indicates the presence of saponins.

Test for Tannins:

To 2ml of extract 10%alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

Test for Phenols:

To 2ml of extract, 2ml of 5% aqueous ferric chloride was added; formation of blue colour indicates the presence of phenols in the sample extract.

Test for Proteins:

To 2ml of extract, 1ml of 40% sodium hydroxide and few drops of 1%copper sulphate were added; formation of violet colour indicates the presence of peptide linkage molecule in the sample extract.

Test for Cardiac Glycosides:

To 1ml of extract, 0.5ml of glacial acetic acid and 3drops of 1%acqueous ferric chloride solution were added; formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

Test for Carbohydrates:

To 1ml of extract add few drops of Molisch's reagent and then add 1ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour indicates the presence of carbohydrates in the sample extract.

Test for Quinones:

To 2ml of extract 1ml of 5%chloroform and 1ml of 10%ammonia were added; formation of pink violet or red colour indicates the presence of quinones in the sample extract.

Test for steroids:

To 2ml of extract 2ml of acetic anhydride and 1 or 2 drops of concentrated sulphuric acid is added along the sides; the presence of bluish green colour indicates the presence of steroids in the sample extract.

Screening of antimicrobial activity:
Tested organisms:
Gram positive organisms ***staphylococcus aureus, streptococcus pyogenes and*** Gram-negative organisms ***E. Coli, Pseudomonas aeruginosa, Klebsiella pneumonia***.

Agar well diffusion method:
24hrs of fresh inoculums were spread on freshly made nutrient agar plates with sterile glass spreader. After that five wells of 8mm diameter was bored on culture plates with borer, four wells for aliquots of husk extract with different concentrates (50µl, 100µl, 150µl and 200µl) and one for the standard solution. Each well filled with 20µl husk extract with different concentrations and the portion of standard solution was filled. The antibiotic (Erythromycin) used as standard. The plates were incubated at 37°c for 24hrs.

RESULTS AND DISCUSSION:
In the present study the result revealed that phytochemical analysis of methanolic coffee husk extract positive for various constituents such as flavonoids, alkaloids, saponins, tannins, phenols and carbohydrates showed in the table no.1. Proteins, Cardiac glycosides, Quinones and Steroids are absent.

According to Twinkle S. Bansode et al (2015) Flavonoids have wide range of biological properties like antimicrobial, antiviral, anti-inflammatory properties. Alkaloids and Phenolic compounds have antidiabetic property. Saponins exhibit various biological activities, help in the lowering serum cholesterol levels. Tannins have reported to have a cardio protective, anti-inflammatory, anti-carcinogenic property. The coffee husk extract quantitative traits like yield of bioactive compounds, can be used in the food and pharmaceutical industries.

Table no 1: Phytochemical analysis

S.No.	Name of the test	Methanol Extract
1	Flavonoids	positive
2	Alkaloids	positive
3	saponins	positive
4	Tannins	positive
5	Phenols	positive
6	Proteins	negative
7	Cardiac Glycosides	negative
8	Carbohydrates	positive
9	Quinones	negative
10	Steroids	negative

The results of the antimicrobial activity ranged from 12mm to 19mm zone of inhibition against all five bacterial pathogens. Minimum zone of inhibition 12mm was observed in 50 µl concentration against ***Klebsiella pneumoniae*** and maximum zone of inhibition 19mm was observed in 200µl concentration against ***E. coli*** shown in the table no 2. Fig no.1 and 2

In the 50µl concentration ***Pseudomonas aeruginosa*** and ***Staphylococcus aureus*** showed lowest zone of inhibition(12mm) and highest zone of inhibition(14mm) was observed against ***E. coli*** and ***Klebsiella pneumoniae***. The 100µl concentration of extract resulting the same organisms showed minimum(13mm) and maximum(15mm) zone of inhibition.

In the 150µl concentration of extract the lowest zone of inhibition (13mm) was observed against ***Streptococcus pyogenes*** and ***Pseudomonas aeruginosa*** and ***E. coli*** and ***Klebsiella pneumoniae*** presented highest zone of inhibition(18mm).

In the 200µl concentration the minimum zone of inhibition(14mm) was observed against ***Streptococcus pyogenes*** and maximum zone of inhibition(19mm) was observed against ***E. coli***.

Compare to studies of Chalali et al (2015) the extracts showed higher inhibitory effect against gram positive bacteria than gram negative bacteria. In our study gram negative bacteria showed higher inhibitory effect than gram positive bacteria against methanol extract of coffee husk.

Table no 2: zone of inhibition in mm of Methanolic coffee husk Extract

Zone of inhibition in mm					
Micro organism	**50µl**	**100µl**	**150µl**	**200µl**	**contol**
Streptococcus pyogenes	13±0.5	13±0.5	13±0.5	14±0.5	10
Staphylococcus aureus	12±0.5	13±0.5	14±0	15±0.5	11
E.Coli	14±0.5	15±1	18±0.5	19±1	12
Klebsiella Pneumoniae	14±0.5	15±0.5	15±1	17±0	10
Pseudomonas aeroginosa	12±1	13±0.5	13±0.5	15±0.5	10

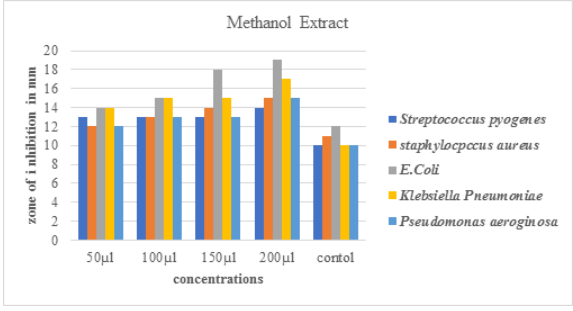

Figure no.1 Anti microbial activity of husk extract

CONCLUSION:
From the above observations, the study concluded the coffee husk extract has rich source of phytochemicals, and also pharmaceutical significance. The extract showed higher inhibitory effect on gram negative bacteria (E. coli and Klebsiella Pneumoniae). In future, the use of coffee husk could be interesting to evaluate the economic and environmental impact as source of bioactive compounds. Further study is required to find their potentials such as anti-oxidant and GCMS analysis.

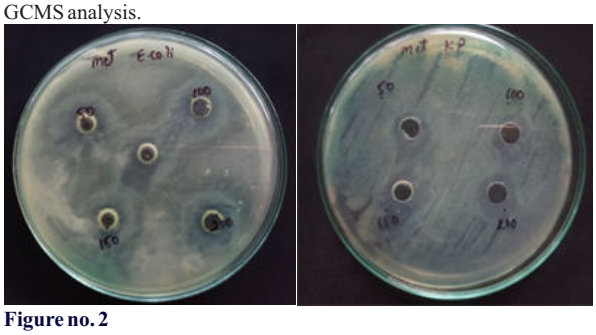

Figure no. 2
Methanol Extract of coffee husk extract showing zone of inhibition against ***E. Coli*** and ***K.Pneumoniae***

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